

PYRAZOLOANTHRONE AND DERIVATIVES THEREOF AS JNK INHIBITORS
AND COMPOSITIONS AND METHODS RELATED THERETO

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims the benefit of U.S. Application No. ~~09/378,234~~ ^{60/240,928} filed August 19, 1999 (incorporated by reference in its entirety), which ~~application was converted to U.S. Provisional Application No. (awaiting) by petition filed August 16, 2000.~~ ^{PROVISIONAL}

TECHNICAL FIELD

10 This invention is generally directed to pyrazoloanthrone and derivatives thereof which have utility over a wide range of indications, including activity as Jun N-terminal kinase inhibitors, and related compositions and methods.

BACKGROUND OF THE INVENTION

15 The Jun N-terminal kinase (JNK) pathway is activated by exposure of cells to environmental stress or by treatment of cells with pro-inflammatory cytokines. Targets of the JNK pathway include the transcription factors c-jun and ATF2 (Whitmarsh A.J., and Davis R.J. *J. Mol. Med.* 74:589-607, 1996). These transcription factors are members of the basic leucine zipper (bZIP) group that bind as homo- and hetero-dimeric complexes to AP-
20 1 and AP-1-like sites in the promoters of many genes (Karin M., Liu Z.G. and Zandi E. *Curr Opin Cell Biol* 9:240-246, 1997). JNK binds to the N-terminal region of c-jun and ATF-2 and phosphorylates two sites within the activation domain of each transcription factor (Hibi M., Lin A., Smeal T., Minden A., Karin M. *Genes Dev.* 7:2135-2148, 1993; Mohit A.A., Martin M.H., and Miller C.A. *Neuron* 14:67-75, 1995). Three JNK enzymes
25 have been identified as products of distinct genes (Hibi et al, *supra*; Mohit et al., *supra*). Ten different isoforms of JNK have been identified. These represent alternatively spliced forms of three different genes: JNK1, JNK2 and JNK3. JNK1 and 2 are ubiquitously expressed in human tissues, whereas JNK3 is selectively expressed in the brain, heart and testis (Dong, C., Yang, D., Wysk, M., Whitmarsh, A., Davis, R., Flavell, R. *Science* 270:1-

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4, 1998). Gene transcripts are alternatively spliced to produce four-JNK1 isoforms, four-JNK2 isoforms and two-JNK3 isoforms. JNK1 and 2 are expressed widely in mammalian tissues, whereas JNK3 is expressed almost exclusively in the brain. Selectivity of JNK signaling is achieved via specific interactions of JNK pathway components and by use of scaffold proteins that selectively bind multiple components of the signaling cascade. JIP-1 (JNK-interacting protein-1) selectively binds the MAPK module, $MLK \rightarrow JNKK1 \rightarrow JNK$.^{12,13} It has no binding affinity for a variety of other MAPK cascade enzymes. Different scaffold proteins are likely to exist for other MAPK signaling cascades to preserve substrate specificity.

JNKs are activated by dual phosphorylation on Thr-183 and Tyr-185. JNKK1 (also known as MKK 4) and JNKK2 (MKK7), two MAPKK level enzymes, can mediate JNK activation in cells (Lin A., Minden A., Martinetto H., Claret F.-Z., Lange-Carter C., Mercurio F., Johnson G.L., and Karin M. *Science* 268:286-289, 1995; Tournier C., Whitmarsh A.J., Cavanagh J., Barrett T., and Davis R.J. *Proc. Nat. Acad. Sci. USA* 94:7337-7342, 1997). JNKK2 specifically phosphorylates JNK, whereas JNKK1 can also phosphorylate and activate p38. Both JNKK1 and JNKK2 are widely expressed in mammalian tissues. JNKK1 and JNKK2 are activated by the MAPKKK enzymes, MEKK1 and 2 (Lange-Carter C.A., Pleiman C.M., Gardner A.M., Blumer K.J., and Johnson G.L. *Science* 260:315-319, 1993; Yan M., Dai J.C., Deak J.C., Kyriakis J.M., Zon L.I., Woodgett J.R., and Templeton D.J. *Nature* 372:798-781, 1994). Both MEKK1 and MEKK2 are widely expressed in mammalian tissues.

Activation of the JNK pathway has been documented in a number of disease settings, providing the rationale for targeting this pathway for drug discovery. In addition, molecular genetic approaches have validated the pathogenic role of this pathway in several diseases. For example, autoimmune and inflammatory diseases arise from the over-activation of the immune system. Activated immune cells express many genes encoding inflammatory molecules, including cytokines, growth factors, cell surface receptors, cell adhesion molecules and degradative enzymes. Many of these genes are regulated by the JNK pathway, through activation of the transcription factors AP-1 and ATF-2, including

TNF α , IL-2, E-selectin and matrix metalloproteinases such as collagenase-1 (Manning A.M. and Mercurio F. *Exp Opin Invest Drugs* 6: 555-567, 1997). Monocytes, tissue macrophages and tissue mast cells are key sources of TNF α production. The JNK pathway regulates TNF α production in bacterial lipopolysaccharide-stimulated macrophages, and in
 5 mast cells stimulated through the Fc ϵ RII receptor (Swantek J.L., Cobb M.H., Geppert T.D. *Mol. Cell. Biol.* 17:6274-6282, 1997; Ishizuka, T., Tereda N., Gerwins, P., Hamelmann E., Oshiba A., Fanger G.R., Johnson G.L., and Gelfland E.W. *Proc. Nat. Acad. Sci. USA* 94:6358-6363, 1997). Inhibition of JNK activation effectively modulates TNF α secretion from these cells. The JNK pathway therefore regulates production of this key pro-
 10 inflammatory cytokine. Matrix metalloproteinases (MMPs) promote cartilage and bone erosion in rheumatoid arthritis, and generalized tissue destruction in other autoimmune diseases. Inducible expression of MMPs, including MMP-3 and MMP-9, type II and IV collagenases, are regulated via activation of the JNK pathway and AP-1 (Gum, R., Wang, H., Lengyel, E., Juarez, J., and Boyd, D. *Oncogene* 14:1481-1493, 1997). In human
 15 rheumatoid synoviocytes activated with TNF α , IL-1, or Fas ligand the JNK pathway is activated (Han Z., Boyle D.L., Aupperle K.R., Bennett B., Manning A.M., Firestein G.S. *J. Pharm. Exp. Therap.* 291:1-7, 1999; Okamoto K., Fujisawa K., Hasunuma T., Kobata T., Sumida T., and Nishioka K. *Arth & Rheum* 40: 919-926, 1997). Inhibition of JNK activation results in decreased AP-1 activation and collagenase-1 expression (Han et al.,
 20 *supra*). The JNK pathway therefore regulates MMP expression in cells involved in rheumatoid arthritis.

Inappropriate activation of T lymphocytes initiates and perpetuates many autoimmune diseases, including asthma, inflammatory bowel disease and multiple sclerosis. The JNK pathway is activated in T cells by antigen stimulation and CD28
 25 receptor co-stimulation and regulates production of the growth factor IL-2 and cellular proliferation (Su B., Jacinto E., Hibi M., Kallunki T., Karin M., Ben-Neriah Y. *Cell* 77:727-736, 1994; Faris M., Kokot N., Lee L., and Nel A.E. *J. Biol. Chem.* 271:27366-27373, 1996). Peripheral T cells from mice genetically deficient in JNKK1 show decreased proliferation and IL-2 production after CD28 co-stimulation and PMA / Ca²⁺ ionophore

activation, providing important validation for the role of the JNK pathway in these cells (Nishina H., Bachmann M., Oliveria-dos-Santos A.J., et al. *J. Exp. Med.* 186: 941-953, 1997). It is known that T cells activated by antigen receptor stimulation in the absence of accessory cell-derived co-stimulatory signals lose the capacity to synthesize IL-2, a state called clonal anergy. This is an important process by which auto-reactive T cell populations are eliminated from the peripheral circulation. Of note, anergic T cells fail to activate the JNK pathway in response to CD3- and CD28-receptor co-stimulation, even though expression of the JNK enzymes is unchanged (Li W., Whaley C.D., Mondino A., and Mueller D.L. *Science* 271: 1272-1276, 1996). Recently, the examination of JNK-deficient mice revealed that the JNK pathway plays a key role in T cell activation and differentiation to T helper 1 and 2 cell types. JNK 1 or JNK2 knockout mice develop normally and are phenotypically unremarkable. Activated naïve CD4⁺ T cells from these mice fail to produce IL-2 and do not proliferate well (Sabapathy, K, Hu, Y, Kallunki, T, Schreiber, M, David, J-P, Jochum, W, Wagner, E, Karin, M. *Curr Biol* 9: 116-125, 1999). It is possible to induce T cell differentiation in T cells from these mice, generating Th1 cells (producers of IFN- γ and TNF β) and Th2 effector cells (producers of IL-4, IL-5, IL-6, IL-10 and IL-13) [22,23]. Deletion of either JNK1 or JNK2 in mice resulted in a selective defect in the ability of Th1 effector cells to express IFN γ . This suggests that JNK1 and JNK2 do not have redundant functions in T cells and that they play different roles in the control of cell growth, differentiation and death. The JNK pathway therefore, is an important point for regulation of T cell responses to antigen.

Cardiovascular disease (CVD) accounts for nearly one quarter of total annual deaths worldwide. Vascular disorders such as atherosclerosis and restenosis result from dysregulated growth of the vessel wall, restricting blood flow to vital organs. The JNK pathway is activated by atherogenic stimuli and regulates local cytokine and growth factor production in vascular cells (Yang, DD, Conze, D, Whitmarsh, AJ, et al, *Immunity*, 9:575, 1998). In addition, alterations in blood flow, hemodynamic forces and blood volume lead to JNK activation in vascular endothelium, leading to AP-1 activation and pro-

atherosclerotic gene expression (Aspenstrom P., Lindberg U., and Hall A. *Curr. Biol.* 6:70-77, 1996). Ischemia and ischemia coupled with reperfusion in the heart, kidney or brain results in cell death and scar formation, which can ultimately lead to congestive heart failure, renal failure or cerebral dysfunction. In organ transplantation, reperfusion of previously ischemic donor organs results in acute leukocyte-mediated tissue injury and delay of graft function. The JNK pathway is activated by ischemia and reperfusion (Li Y., Shyy J., Li S., Lee J., Su B., Karin M., Chien S *Mol. Cell. Biol.* 16:5947-5954, 1996), leading to the activation of JNK-responsive genes and leukocyte-mediated tissue damage. In a number of different settings JNK activation can be either pro- or anti-apoptotic. JNK activation is correlated with enhanced apoptosis in cardiac tissues following ischemia and reperfusion (Pombo CM, Bonventre JV, Avruch J, Woodgett JR, Kyriakis J.M, Force T. *J. Biol. Chem.* 269:26546-26551, 1994).

Cancer is characterized by uncontrolled growth, proliferation and migration of cells. Cancer is the second leading cause of death with 500,000 deaths and an estimated 1.3 million new cases in the United States in 1996. The role of signal transduction pathways contributing to cell transformation and cancer is a generally accepted concept. The JNK pathway leading to AP-1 appears to play a critical role in cancer. Expression of c-jun is altered in early lung cancer and may mediate growth factor signaling in non-small cell lung cancer (Yin T., Sandhu G., Wolfgang C.D., Burrier A., Webb R.L., Rigel D.F. Hai T., and Whelan J. *J. Biol. Chem.* 272:19943-19950, 1997). Indeed, over-expression of c-jun in cells results in transformation, and blocking c-jun activity inhibits MCF-7 colony formation (Szabo E., Riffe M., Steinberg S.M., Birrer M.J., Linnoila R.I. *Cancer Res.* 56:305-315, 1996). DNA-damaging agents, ionizing radiation and tumor necrosis factor activate the JNK pathway. In addition to regulating c-jun production and activity, JNK activation can regulate phosphorylation of p53, and thus can modulate cell cycle progression (Chen T.K., Smith L.M., Gebhardt D.K., Birrer M.J., Brown P.H. *Mol. Carcinogenesis* 15:215-226, 1996). The oncogene BCR-Abl, associated with t(9,22) Philadelphia chromosome translocation of chronic myelogenous leukemia, activates JNK and leads to transformation of hematopoietic cells (Milne D.M., Campbell L.E., Campbell

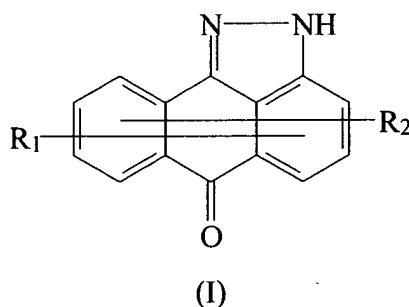
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D.G., Meek D.W. *J. Biol. Chem.* 270:5511-5518, 1995). Selective inhibition of JNK activation by a naturally occurring JNK inhibitory protein, called JIP-1, blocks cellular transformation caused by BCR-Abl expression (Raitano A.B., Halpern J.R., Hambuch T.M., Sawyers C.L. *Proc. Nat. Acad. Sci USA* 92:11746-11750, 1995). Thus, JNK inhibitors may block transformation and tumor cell growth.

Accordingly, there is a need in the art for selective inhibitors of JNK, as well as for methods for preparation thereof, pharmaceutical compositions comprising such inhibitors, and methods of inhibiting JNK's and treating diseases in mammals which are responsive to JNK inhibition. The present invention fulfills these needs, and provides further related advantages.

SUMMARY OF THE INVENTION

In brief, the present invention is directed to compounds having activity as selective inhibitors of JNK, as well as to compositions and methods related thereto. The compounds of the present invention (also referred to herein as "JNK inhibitors") may generally be classified as "pyrazoloanthrone derivatives" having the following structure (I):



wherein R₁ and R₂ are as defined below, including pharmaceutically acceptable salts thereof.

The present invention is also directed to methods for treating a variety of conditions by administering an effective amount of a JNK inhibitor to an animal or subject in need thereof (referred to herein as a "patient"), typically a warm-blooded animal

(including a human). Prior to administration, the compounds of this invention are preferably formulated as a pharmaceutical composition which contains an effective dosage amount of one or more JNK inhibitors in combination with one (or more) pharmaceutically acceptable carrier(s). Conditions that may be treated by the compounds of this invention, or a pharmaceutical composition containing the same, include any condition which may benefit from administration of JNK inhibitors, and are particularly useful for the prevention and/or treatment of various diseases including (but not limited to) rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gout, asthma, bronchitis, cystic fibrosis, inflammatory bowel disease, irritable bowel syndrome, mucous colitis, ulcerative colitis, Crohn's disease, gastritis, esophagitis, hepatitis, multiple sclerosis, atherosclerosis, restenosis following angioplasty, left ventricular hypertrophy, myocardial infarction, stroke, ischemic damages to heart, kidney, liver, and brain, transplant rejection, endotoxin shock, psoriasis, eczema, dermatitis, epilepsy, Alzheimer's disease, Huntington's disease, Amyotrophic lateral sclerosis, peripheral neuropathies, spinal cord damage, Parkinson's disease, and cancer.

These and other aspects of this invention will be apparent upon reference to the following detailed description. To that end, certain patent and other documents are cited herein to more specifically set forth various aspects of this invention. Each of these documents are hereby incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the ability of a representative compound of this invention to inhibit IL-2 in Jurkat T-Cell.

Figure 2 illustrates the ability of a representative compound of this invention to inhibit TNF- α in a mouse model of endotoxin shock.

Figure 3 illustrates the ability of a representative compound of this invention to inhibit leukocyte recruitment in rat model for inflamed lung.

Figure 4 illustrates the ability of a representative compound of this invention to inhibit paw swelling (Figure 4A), joint destruction (Figure 4B), transcription factor AP-1

activation (Figure 4C), and expression of MMP-13 (Figure 4D) in a rat model for adjuvant arthritis.

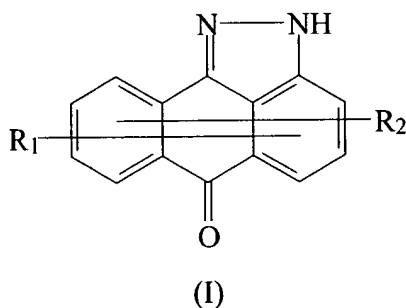
Figure 5 illustrates the ability of a representative compound of this invention to reduce kainic acid-induced seizure response.

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DETAILED DESCRIPTION OF THE INVENTION

As mentioned above, the present invention is directed to compounds which have activity as selective inhibitors of JNK, as well as to compositions and methods relating to the same. The compounds of this invention have the following structure (I):

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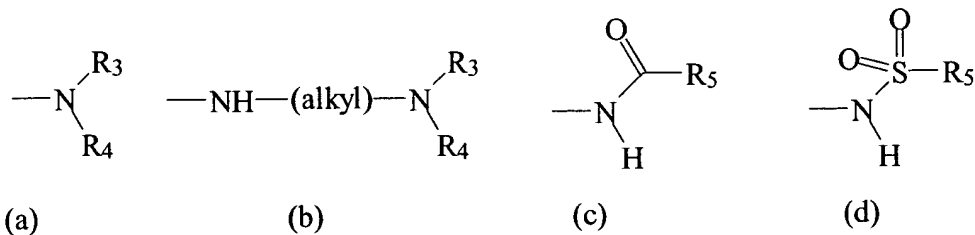


and pharmaceutically acceptable salts thereof, wherein:

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R_1 and R_2 are optional substituents that are the same or different and independently represent alkyl, halogen, nitro, trifluoromethyl, sulfonyl, carboxyl, alkoxycarbonyl, alkoxy, aryl, aryloxy, arylalkyloxy, arylalkyl, cycloalkylalkyloxy, cycloalkyloxy, alkoxyalkyl, alkoxyalkoxy, aminoalkoxy, mono- or di-alkylaminoalkoxy, or a group represented by formula (a), (b), (c) or (d):

20



R₃ and R₄ taken together represent alkylidene or a heteroatom-containing alkylidene, or R₃ and R₄ are the same or different and independently represent hydrogen, alkyl, cycloalkyl, aryl, arylalkyl, cycloalkylalkyl, aryloxyalkyl, alkoxyalkyl, alkoxyamino, or alkoxy(mono- or di-alkylamino); and

R₅ represents hydrogen, alkyl, cycloalkyl, aryl, arylalkyl, cycloalkylalkyl, alkoxy, amino, mono- or di-alkylamino, arylamino, arylalkylamino, cycloalkylamino, or cycloalkylalkylamino.

10

As used herein, the terms used above having following meaning.

“Alkyl” means a straight chain or branched, saturated or unsaturated alkyl chain having from 1 to 8 carbon atoms, such as methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert-butyl, propylenyl, 1-butenyl, propynyl, and the like.

15

“Halogen” means fluorine, chlorine, bromine or iodine.

“Trifluoromethyl” means -CF₃.

“Sulfonyl” means -SO₃H;

“Carboxyl” means -COOH.

“Alkoxy” means -O-(alkyl), such as methoxy, ethoxy, n-propyloxy, iso-propyloxy, n-butyloxy, iso-butyloxy, and the like.

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“Alkoxyalkoxy” means -O-(alkyl)-O-(alkyl), such as -OCH₂CH₂OCH₃, and the like.

“Alkoxycarbonyl” means -C(=O)O-(alkyl), such as -C(=O)OCH₃, -C(=O)OCH₂CH₃, and the like.

25

“Alkoxyalkyl” means -(alkyl)-O-(alkyl), such as -CH₂OCH₃, -CH₂OCH₂CH₃, and the like.

“Aryl” means a carbocyclic or heterocyclic aromatic group containing from 5 to 10 ring atoms. The ring atoms of a carbocyclic aryl group are all carbon atoms, and includes phenyl and naphthyl. The ring atoms of a heterocyclic aryl group contains at least

one heteroatom selected from nitrogen, oxygen and sulfur, and include pyridinyl, pyrimidinyl, furanyl, thienyl, imidazolyl, thiazolyl, pyrazolyl, pyridazinyl, pyrazinyl, triazinyl, tetrazolyl, and indolyl.

“Aryloxy” means -O-(aryl), such as -O-phenyl, -O-pyridinyl, and the like.

5 “Arylalkyl” means -(alkyl)-(aryl), such as benzyl (*i.e.*, -CH₂phenyl), -CH₂-pyridinyl, and the like.

“Arylalkyloxy” means -O-(alkyl)-(aryl), such as -O-benzyl, -O-CH₂-pyridinyl, and the like.

10 “Cycloalkyl” means a cyclic alkyl having from 3 to 7 carbon atoms, such as cyclopropyl, cyclopentyl, cyclohexyl, and the like.

“Cycloalkyloxy” means -O-(cycloalkyl), such as -O-cyclohexyl, and the like.

“Cycloalkylalkyloxy” means -O-(alkyl)-(cycloalkyl), such as -OCH₂cyclohexyl, and the like.

15 “Alkylidene” means the divalent radical -C_nH_{2n}-, wherein n is an integer from 1 to 8, such as -CH₂-, -CH₂CH₂-, -CH₂-CH₂-CH₂-, -CH₂CH₂CH₂CH₂-, -CH₂CH₂CH₂CH₂CH₂-, and the like.

“Heteroatom-containing alkylidene” means an alkylidene wherein at least one carbon atom is replaced by a heteroatom selected from nitrogen, oxygen or sulfur, such as -CH₂CH₂OCH₂CH₂-, and the like.

20 “Aminoalkoxy” means -O-(alkyl)-NH₂, such as -OCH₂NH₂, -OCH₂CH₂NH₂, and the like.

“Mono- or di-alkylamino” means -NH(alkyl) or -N(alkyl)(alkyl), respectively, such as -NHCH₃, -N(CH₃)₂, and the like.

25 “Mono- or di-alkylaminoalkoxy” means -O-(alkyl)-NH(alkyl) or -O-(alkyl)-N(alkyl)(alkyl), respectively, such as -OCH₂NHCH₃, -OCH₂CH₂N(CH₃)₂, and the like.

“Arylamino” means -NH(aryl), such as -NH-phenyl, -NH-pyridinyl, and the like.

“Arylalkylamino” means -NH-(alkyl)-(aryl), such as -NH-benzyl, -NHCH₂-pyridinyl, and the like.

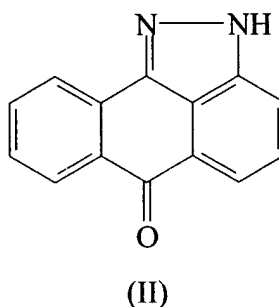
“Alkylamino” means -NH(alkyl), such as -NHCH₃, -NHCH₂CH₃, and the like.

“Cycloalkylamino” means -NH-(cycloalkyl), such as -NH-cyclohexyl, and the like.

5 “Cycloalkylalkylamino” -NH-(alkyl)-(cycloalkyl), such as -NHCH₂-cyclohexyl, and the like.

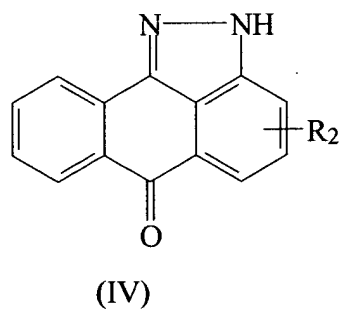
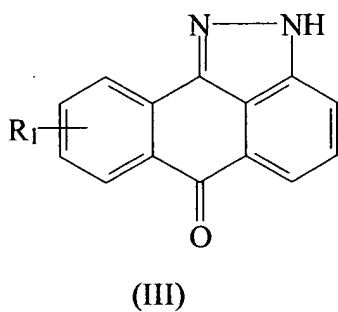
In the embodiment wherein R₁ and R₂ are not present, compounds of this invention have the following structure (II) (also referred to herein as “Compound 1”):

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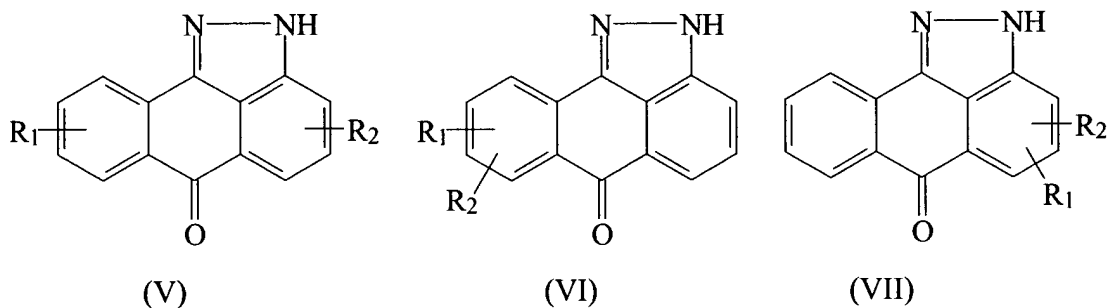
This compound is commercially available from Pfaltz-Bauer (Conn., U.S.).

15 In the embodiment wherein only one of R₁ and R₂ is present, compounds of this invention have one of the following structures (III) or (IV):



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In the embodiment wherein both R_1 and R_2 are present, compounds of this invention have one of the following structures (V), (VI) or (VII):



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Pharmaceutically acceptable salts of compounds of structure (I) are also within the scope of this invention. To this end, the compound may generally be utilized as the free base. Alternatively, the compounds may be used in the form of acid addition salts.

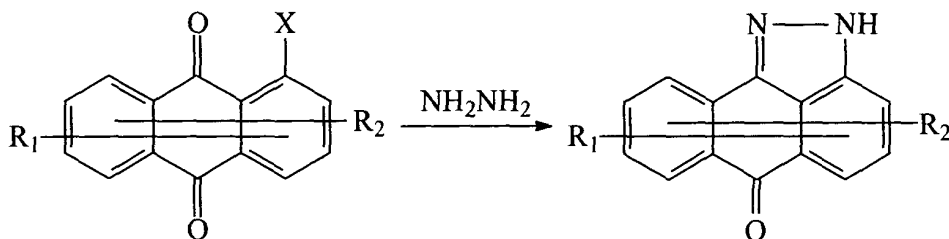
10 Acid addition salts of the free base amino compounds of the present invention may be prepared by methods well known in the art, and may be formed from organic and inorganic acids. Suitable organic acids include maleic, fumaric, benzoic, ascorbic, succinic, methanesulfonic, acetic, oxalic, propionic, tartaric, salicylic, citric, gluconic, lactic, mandelic, cinnamic, aspartic, stearic, palmitic, glycolic, glutamic, and benzenesulfonic

15 acids. Suitable inorganic acids include hydrochloric, hydrobromic, sulfuric, phosphoric, and nitric acids. Thus, the term "pharmaceutically acceptable salt" of a compound of structure (I) is intended to encompass any and all acceptable salt forms.

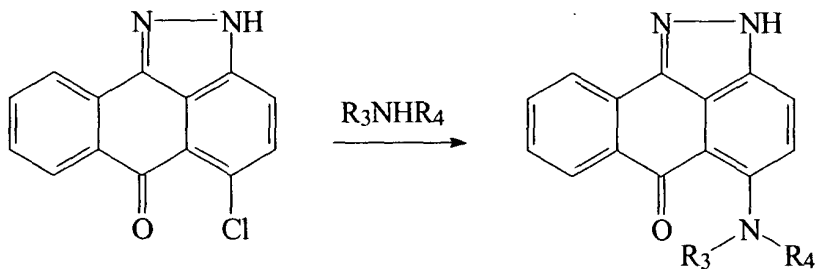
The compounds of this invention may generally be made by organic synthesis techniques known to those skilled in the art, as well as by the following general

20 techniques and by the procedures set forth in the Examples. To that end, the compounds of this invention may be made according to the following Reaction Schemes 1 through 7.

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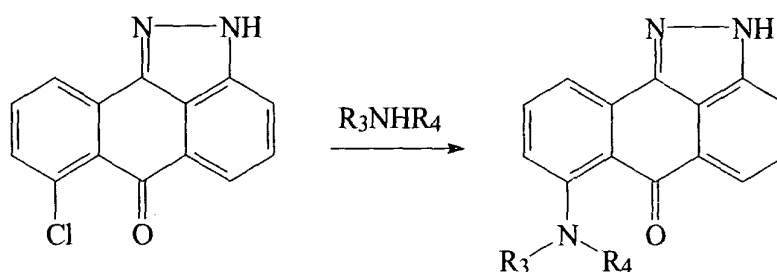
Reaction Scheme 1

- 5 In Reaction Scheme 1, pyrazoloanthrones of this invention may be prepared by condensation of appropriate anthraquinones having a leaving group at the 1-position (such as fluoro, chloro, bromo, iodo, nitro, methanesulfonyloxy, tosyloxy or phenoxy) with hydrazine in a suitable solvent (such as pyridine, dimethylformamide, methylene chloride, chloroform, or dioxane). The reaction is carried out at temperatures ranging 0°C to 200°C
- 10 for 1 to 16 hours. Suitable anthraquinone starting materials are commercially available from a variety of sources with the R_1 and/or R_2 groups at various positions on the anthraquinone ring. For purpose of illustration, the following reaction schemes depict synthesis of 5- and/or 7-substituted pyrazoloanthrones. One skilled in the art will recognize that pyrazoloanthrones substituted at other positions may be made in a similar manner from
- 15 the appropriately substituted pyrazoloanthrone starting material.

Reaction Scheme 2

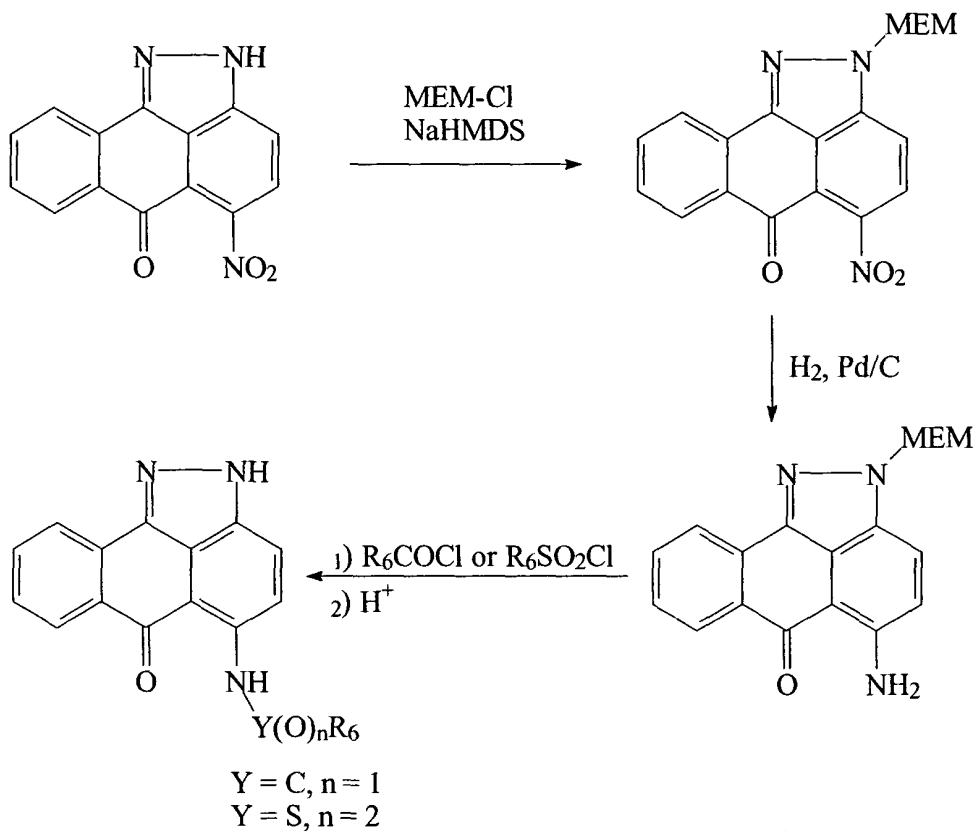
In Reaction Scheme 2, pyrazoloanthrones with 5-amino substituents may be prepared by condensation of 5-chloropyrazoloanthrone with mono- or disubstituted amines at 0 to 250°C for 1 to 16 hours, either in the absence or the presence of a solvent. Typically solvents are pyridine, dimethylformamide, dimethylsulfoxide, dichloroethane, chloroform, tetrahydrofuran, dioxane, diglyme, or triglyme in the presence of excess amount of the amine, or in the presence of an acid quenching agent such as triethylamine, diisopropylethylamine, sodium bicarbonate, potassium carbonate, or sodium hydroxide.

Reaction Scheme 3



In Reaction Scheme 3, pyrazoloanthrones with 7-amino substituents may be prepared by condensation of 7-chloropyrazoloanthrone with mono- or disubstituted amines at 0 to 250°C for 1 to 16 hours either in the absence or the presence of a solvent. Typically solvents are pyridine, dimethylformamide, dimethylsulfoxide, dichloroethane, chloroform, tetrahydrofuran, dioxane, diglyme, or triglyme in the presence of excess amount of the amine, or in the presence of an acid quenching agent such as triethylamine, diisopropylethylamine, sodium bicarbonate, potassium carbonate, or sodium hydroxide.

Reaction Scheme 4

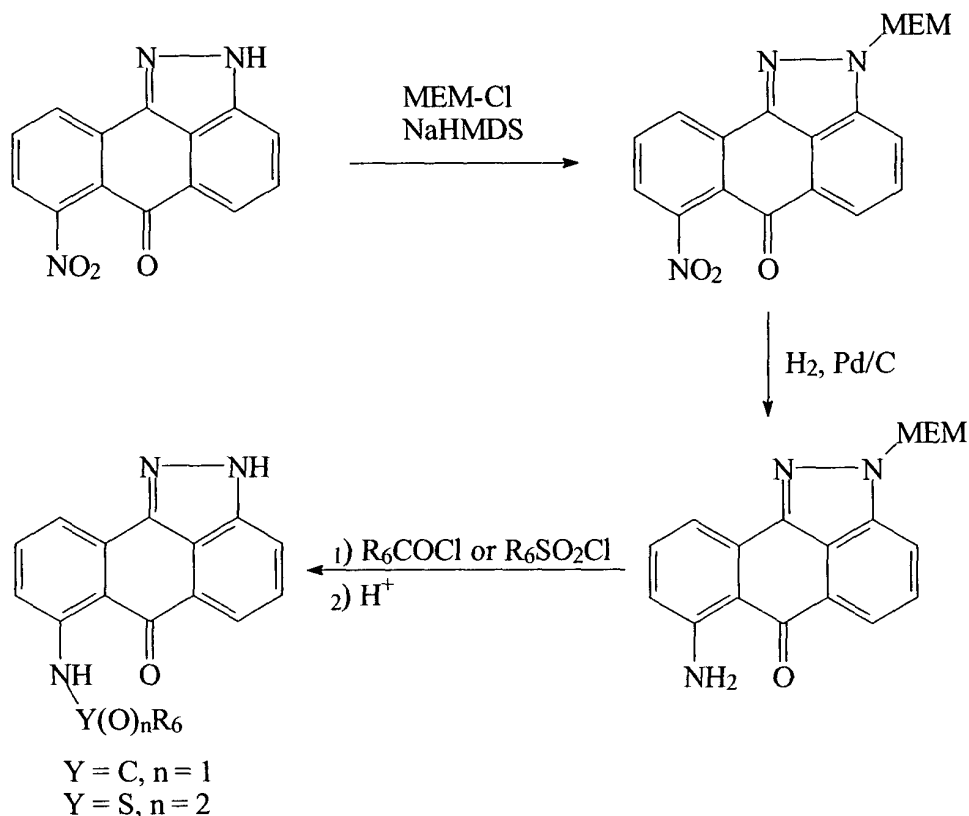


- 5 In Reaction Scheme 4, pyrazoloanthrones with 5-acyl- or sulfonylamino substituents may be prepared by condensation of 5-amino-2-(2-methoxyethoxymethyl)pyrazoloanthrone with acid chlorides and sulfonyl chlorides followed by the deprotection. Condensation of 5-amino-2-(2-methoxyethoxymethyl)pyrazoloanthrone with appropriate acid chlorides R_6COCl or
- 10 sulfonyl chlorides $\text{R}_6\text{SO}_2\text{Cl}$ is carried out in the presence of an acid quenching agent such as triethylamine, diisopropylethylamine, sodium bicarbonate, potassium carbonate, or sodium hydroxide at -20 to 50°C for 0.5 to 16 hours in solvents such as methylene chloride, chloroform, tetrahydrofuran, dioxane, dimethylformamide, and ethyl acetate. The deprotection step may be performed by the treatment of the product mentioned above with

an acid such as trifluoroacetic acid, aqueous hydrochloric acid, aqueous hydrobromic acid, or aqueous sulfuric acid.

The starting material may be prepared in two steps. The 2-position of 5-nitropyrazoloanthrone may be protected by a protective group such as methoxymethyl (MOM), methoxyethoxymethyl (MEM), 2-trimethylsilylethoxymethyl (SEM), or 4-methoxybenzyl (PMB) with an aid of a base such as triethylamine, diisopropylethylamine, pyridine, sodium hexamethyldisilazide, potassium hexamethyldisilazide, or lithium diisopropylamide. 4-(N, N-dimethylamino)pyridine (DMAP) may be used as a catalyst when a tertiary amine is used as a base. The reaction is typically carried out at -40 to 60°C for 1 to 16 hours in a solvent such as methylene chloride, chloroform, tetrahydrofuran, dioxane, or dimethoxyethane. As the nitrogen protective group, MEM group is preferred.

N-Protected 5-nitropyrazoloanthrone is then reduced to its 5-amino derivative by a variety of reducing agents such as Sn or Fe metal in acidic media such as acetic acid or aqueous hydrochloric acid. The reaction is typically run at 20 to 160°C for 1 to 16 hours. The same transformation can be carried out by hydrogenation in the presence of a transition-metal catalyst such as palladium, platinum, rhodium, or iridium with or without a support such as charcoal in a solvent such as ethanol, ethyl acetate, tetrahydrofuran, dioxane, or dimethoxyethane at 1 to 20 atmospheres of hydrogen at 20 to 60°C for 1 to 16 hours. The procedure using hydrogenation with palladium on charcoal as the catalyst is preferred.

Reaction Scheme 5

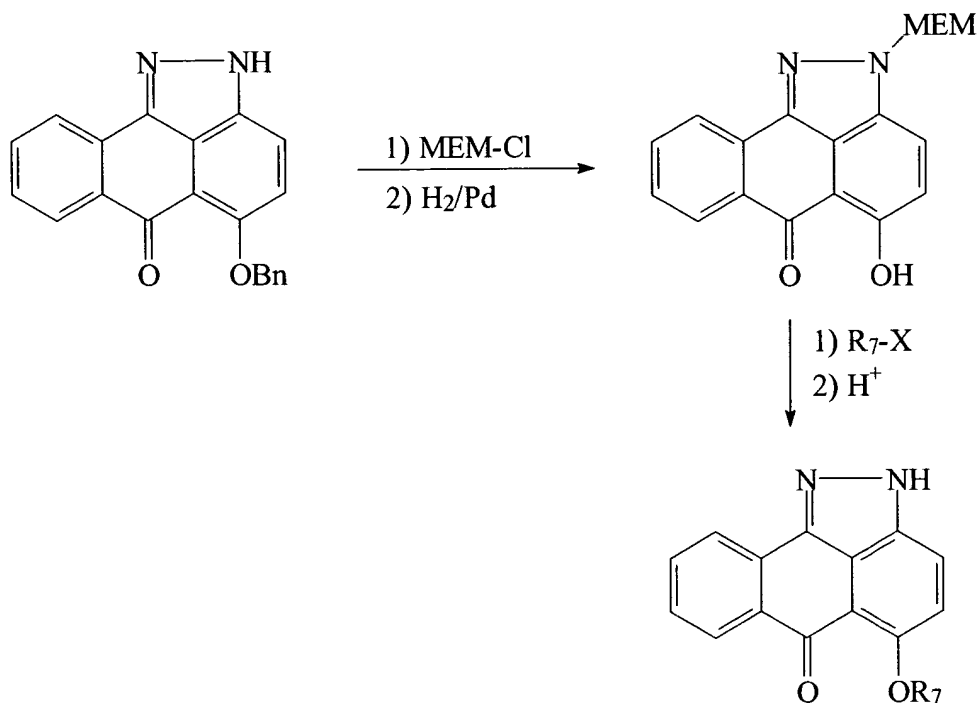
- 5 In Reaction Scheme 5, pyrazoloanthrones with 7-acyl- or sulfonylamino substituents may be prepared by condensation of 7-amino-2-(2-methoxyethoxymethyl)pyrazoloanthrone with acid chlorides and sulfonyl chlorides followed by the deprotection. Condensation of 7-amino-2-(2-methoxyethoxymethyl)pyrazoloanthrone with appropriate acid chlorides R_6COCl or
- 10 sulfonyl chlorides $\text{R}_6\text{SO}_2\text{Cl}$ is carried out in the presence of an acid quenching agent such as triethylamine, diisopropylethylamine, sodium bicarbonate, potassium carbonate, or sodium hydroxide at -20 to 50°C for 0.5 to 16 hours in solvents such as methylene chloride, chloroform, tetrahydrofuran, dioxane, dimethylformamide, or ethyl acetate. The deprotection step may be performed by the treatment of the product mentioned above with

an acid such as trifluoroacetic acid, aqueous hydrochloric acid, aqueous hydrobromic acid, or aqueous sulfuric acid.

The starting material is prepared in two steps. The 2-position of 7-nitropyrzoloanthrone is protected by a protective group such as methoxymethyl (MOM), methoxyethoxymethyl (MEM), 2-trimethylsilylethoxymethyl (SEM), or 4-methoxybenzyl (PMB) with an aid of a base such as triethylamine, diisopropylethylamine, pyridine, sodium hexamethyldisilazide, potassium hexamethyldisilazide, or lithium diisopropylamide. 4-(N, N-dimethylamino)pyridine (DMAP) can be used as a catalyst when a tertiary amine is used as a base. The reaction is typically carried out at -40 to 60°C for 1 to 16 hours in a solvent such as methylene chloride, chloroform, tetrahydrofuran, dioxane, or dimethoxyethane. As the nitrogen protective group, MEM group is preferred.

N-Protected 7-nitropyrzoloanthrone is then reduced to its 7-amino derivative by a variety of reducing agents such as Sn or Fe metal in acidic media such as acetic acid or aqueous hydrochloric acid. The reaction is typically run at 20 to 160°C for 1 to 16 hours. The same transformation can be carried out by hydrogenation in the presence of a transition-metal catalyst such as palladium, platinum, rhodium, or iridium with or without a support such as charcoal in a solvent such as ethanol, ethyl acetate, tetrahydrofuran, dioxane, or dimethoxyethane at 1 to 20 atmospheres of hydrogen at 20 to 60°C for 1 to 16 hours. The procedure using hydrogenation with palladium on charcoal as the catalyst is preferred.

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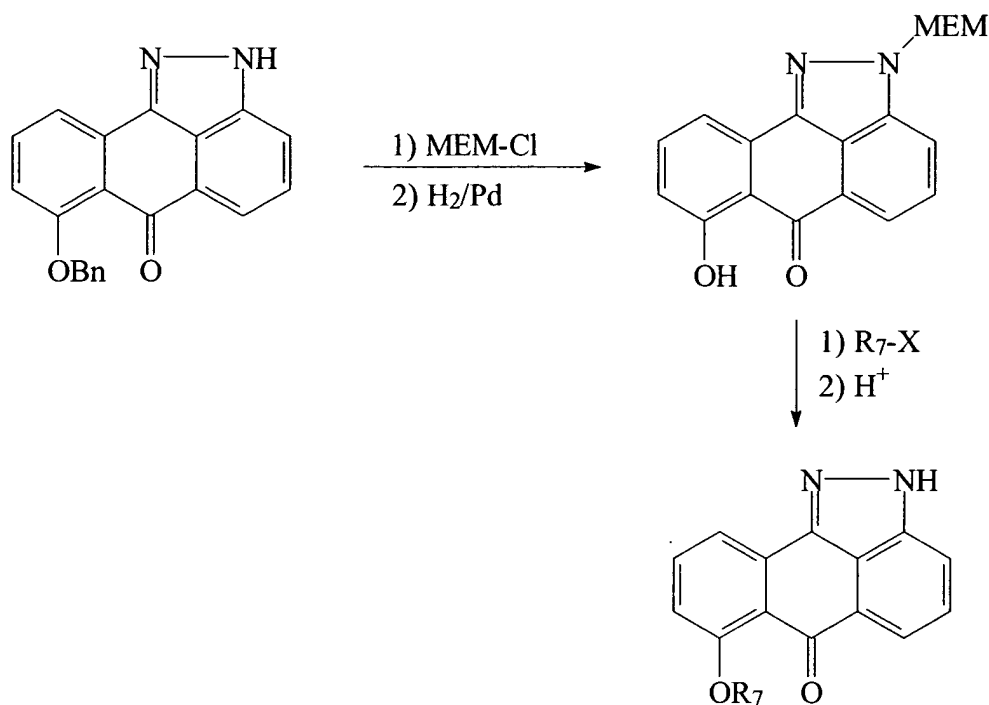
Reaction Scheme 6

- 5 In Reaction Scheme 6, pyrazoloanthrones with 5-alkoxy substituents may be prepared by condensation of 5-hydroxy-2-(2-methoxyethoxymethyl)-pyrazoloanthrone with alkyl halides and sulfonates R₇-X followed by the deprotection. As the leaving group X, chloride, bromide, iodide, methanesulfonate, tosylate, benzenesulfonate, or triflate can be used. Condensation of 5-hydroxy-2-(2-methoxyethoxymethyl)pyrazoloanthrone with
- 10 appropriate alkyl halides and sulfonates is carried out in the presence of an acid quenching agent such as triethylamine, diisopropylethylamine, sodium bicarbonate, potassium carbonate, or sodium hydroxide at -20 to 50°C for 0.5 to 16 hours in solvents such as methylene chloride, chloroform, tetrahydrofuran, dioxane, dimethylformamide, or ethyl acetate. The deprotection step is performed by the treatment of the product mentioned
- 15 above with an acid such as trifluoroacetic acid, aqueous hydrochloric acid, aqueous hydrobromic acid, or aqueous sulfuric acid.

The starting material is prepared in two steps. The 2-position of 5-benzyloxypyrazoloanthrone is protected by a protective group such as methoxymethyl (MOM), methoxyethoxymethyl (MEM), 2-trimethylsilylethoxymethyl (SEM), or 4-methoxybenzyl (PMB) with an aid of a base such as triethylamine, diisopropylethylamine, 5 pyridine, sodium hexamethyldisilazide, potassium hexamethyldisilazide, or lithium diisopropylamide. 4-(N, N-dimethylamino)pyridine (DMAP) can be used as a catalyst when a tertiary amine is used as a base. The reaction is typically carried out at -40 to 60°C for 1 to 16 hours in a solvent such as methylene chloride, chloroform, tetrahydrofuran, dioxane, or dimethoxyethane. As the nitrogen protective group, MEM group is preferred.

10 N-Protected 5-benzyloxypyrazoloanthrone is then reduced to its 5-hydroxy derivative by hydrogenation in the presence of a transition-metal catalyst, such as palladium platinum, rhodium, or iridium with or without a support such as charcoal in a solvent such as ethanol, ethyl acetate, tetrahydrofuran, dioxane, or dimethoxyethane at 1 to 20 atmospheres of hydrogen at 20 to 60°C for 1 to 16 hours. The procedure using 15 hydrogenation with palladium on charcoal as the catalyst is preferred.

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Reaction Scheme 7

- 5 In Reaction Scheme 7, pyrazoloanthrones with 5-alkoxy substituents may be prepared by condensation of 7-hydroxy-2-(2-methoxyethoxymethyl)-pyrazoloanthrone with alkyl halides and sulfonates R₇-X followed by the deprotection. As the leaving group X, chloride, bromide, iodide, methanesulfonate, tosylate, benzenesulfonate, or triflate can be used. Condensation of 7-hydroxy-2-(2-methoxyethoxymethyl)pyrazoloanthrone with
- 10 appropriate alkyl halides and sulfonates is carried out in the presence of an acid quenching agent such as triethylamine, diisopropylethylamine, sodium bicarbonate, potassium carbonate, or sodium hydroxide at -20 to 50°C for 0.5 to 16 hours in solvents such as methylene chloride, chloroform, tetrahydrofuran, dioxane, dimethylformamide, or ethyl acetate. The deprotection step is performed by the treatment of the product mentioned
- 15 above with an acid such as trifluoroacetic acid, aqueous hydrochloric acid, aqueous hydrobromic acid, or aqueous sulfuric acid.

The starting material is prepared in two steps. The 2-position of 7-benzyloxypyrazoloanthrone is protected by a protective group such as methoxymethyl (MOM), methoxyethoxymethyl (MEM), 2-trimethylsilylethoxymethyl (SEM), or 4-methoxybenzyl (PMB) with an aid of a base such as triethylamine, diisopropylethylamine, pyridine, sodium hexamethyldisilazide, potassium hexamethyldisilazide, or lithium diisopropylamide. 4-(N, N-dimethylamino)pyridine (DMAP) can be used as a catalyst when a tertiary amine is used as a base. The reaction is typically carried out at -40 to 60°C for 1 to 16 hours in a solvent such as methylene chloride, chloroform, tetrahydrofuran, dioxane, or dimethoxyethane. As the nitrogen protective group, MEM group is preferred.

10 N-Protected 7-benzyloxypyrazoloanthrone is then reduced to its 7-hydroxy derivative by hydrogenation in the presence of a transition-metal catalyst, such as palladium platinum, rhodium, or iridium with or without a support such as charcoal in a solvent such as ethanol, ethyl acetate, tetrahydrofuran, dioxane, or dimethoxyethane at 1 to 20 atmospheres of hydrogen at 20 to 60°C for 1 to 16 hours. The procedure using 15 hydrogenation with palladium on charcoal as the catalyst is preferred.

Compounds of structures (V), (VI) and (VII) may be made by the same procedures as outlined above by utilizing starting materials having multiple reactive sites at the corresponding positions to the desired product.

In another embodiment of the invention, pharmaceutical compositions 20 containing one or more compounds of this invention are disclosed. For purpose of administration, a compound of structure (I) is preferably formulated as a pharmaceutical composition. Pharmaceutical compositions of the present invention comprise a compound of this invention and a pharmaceutically acceptable carrier, wherein the compound is present in the composition in an amount which is effective to treat the condition of interest. 25 Preferably, the pharmaceutical compositions of the present invention include a compound of structure (I) in an amount from 0.1 mg to 250 mg per dosage depending upon the route of administration, and more typically from 1 mg to 60 mg. Appropriate concentrations and dosages can be readily determined by one skilled in the art.

Pharmaceutically acceptable carriers are familiar to those skilled in the art. For compositions formulated as liquid solutions, acceptable carriers include saline and sterile water, and may optionally include antioxidants, buffers, bacteriostats and other common additives. The compositions can also be formulated as pills, capsules, granules, or tablets which contain, in addition to a compound of this invention, diluents, dispersing and surface active agents, binders, and lubricants. One skilled in this art may further formulate the compounds of this invention in an appropriate manner, and in accordance with accepted practices, such as those disclosed in *Remington's Pharmaceutical Sciences*, Gennaro, Ed., Mack Publishing Co., Easton, PA 1990.

10 In another embodiment, the present invention provides a method for treating a variety of conditions by administering an effective amount of a JNK inhibitor to a patient in need thereof. Conditions that may be treated by the compounds of this invention, or a pharmaceutical composition containing the same, include any condition which is responsive to JNK inhibition, and thereby benefit from administration of a JNK inhibitor.

15 Representative conditions in this regard include (but not limited to) rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gout, asthma, bronchitis, cystic fibrosis, inflammatory bowel disease, irritable bowel syndrome, mucous colitis, ulcerative colitis, Crohn's disease, gastritis, esophagitis, hepatitis, multiple sclerosis, atherosclerosis, restenosis following angioplasty, left ventricular hypertrophy, myocardial infarction, stroke,

20 ischemic damage to the heart, kidney, liver, or brain, transplant rejection (such as kidney, liver, heart, lung, and the like), endotoxin shock, psoriasis, eczema, dermatitis, epilepsy, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, peripheral neuropathies, spinal cord damage, and cancer.

The methods of this invention include systemic administration of a compound of this invention, preferably in the form of a pharmaceutical composition. As used herein, systemic administration encompasses both oral and parenteral methods of administration. For oral administration, suitable pharmaceutical compositions include powders, granules, pills, tablets, and capsules as well as liquids, syrups, suspensions, and emulsions. These compositions may also include flavorants, preservatives, suspending,

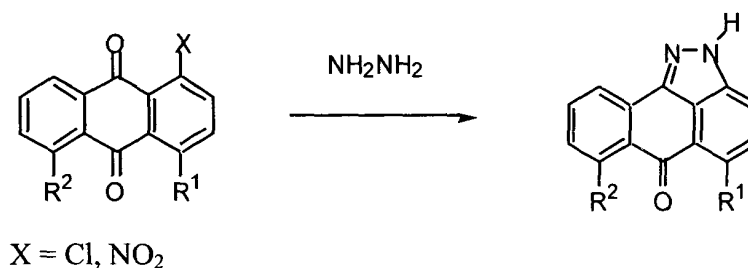
thickening and emulsifying agents, and other pharmaceutically acceptable additives. For parental administration, the compounds of the present invention can be prepared in aqueous injection solutions which may contain buffers, antioxidants, bacteriostats, and other additives commonly employed in such solutions.

5 The following examples are offered by way of illustration, not limitation.

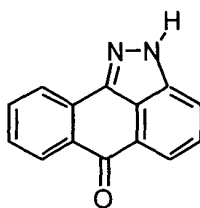
EXAMPLES

EXAMPLE 1

Synthesis of Representative Compounds



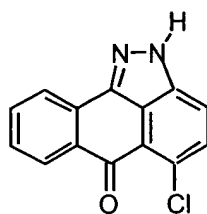
A. Anthra[1,9cd]pyrazol-6(2H)-one ("Compound 1")



20 Anhydrous hydrazine is added to a solution of 2-chloroanthraquinone (Aldrich) in 10 mL pyridine, and the mixture heated at 100°C for 16 hours. The mixture is cooled and the solvent is evaporated in vacuo. The residue is taken in hot 6N HCl, and the solid is collected by filtration. Flash chromatography of the crude material on silica gel affords anthra[1,9cd]pyrazol-6(2H)-one ("Compound 1") as yellow solids.

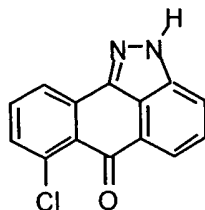
Due to limited solubility of Compound 1, purification of the same may be achieved by first derivatizing Compound 1 to a more soluble intermediate, such as the corresponding acetate, recrystallizing the intermediate, and then converting the intermediate to yield purified Compound 1 in good yield. More specifically, to solution of the pyrazoloanthrone (9.67 g, 43.9 mmol) in acetic acid (700 mL) is added acetic anhydride (12.4 mL, 132 mmol). The solution is heated to 80°C for 5 hours and then cooled to room temperature. After 16 hours, the reaction is cooled to 0°C for 2 hours. The reaction is then filtered to give the N-acetylpyrazoloanthrone intermediate. This intermediate is recrystallized in acetic acid to give the pure intermediate (5.96 g, 52%). ¹H NMR (CDCl₃) δ 10.6 (br s, 1H), 8.46 (d, 1H), 8.33 (d, 1H), 8.26 (d, 1H), 8.08 (d, 1H), 7.96-7.87 (m, 2H), 7.78 (t, 1H), 2.83 (s, 3H); ES-MS (m/z) 263 [M+1]⁺. To a solution of the pure intermediate (5.96 g, 23 mmol) in methanol (600 mL) is added ammonium hydroxide (60 mL). The reaction is stirred at room temperature for 16 hours and then filtered and dried in a vacuum oven. A second crop of crystals is recovered to give a total of 4.8 g of Compound 1 at greater than 98% purity. ES-MS (m/z) 221 [M + 1]⁺.

B. 5-Chloroanthra[1,9cd]pyrazol-6(2H)-one

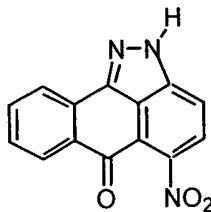


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This compound may be made in the same manner from 1,4-dichloroanthraquinone (commercial product).

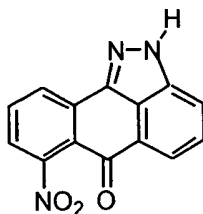
C. 7-Chloroanthra[1,9cd]pyrazol-6(2H)-one

5 This compound may be made in the same manner from 1,5-dichloroanthraquinone (commercial product).

D. 5-Nitroanthra[1,9cd]pyrazol-6(2H)-one

10

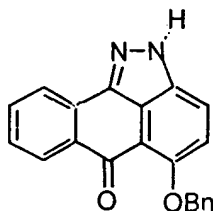
This compound may be made from 1,4-dinitroanthraquinone (Krapcho, A. P.; Avery, K. L., Jr. *J. Org. Chem.* 55, 5562-4, 1990).

15 E. 7-Nitroanthra[1,9cd]pyrazol-6(2H)-one

This compound may be made in the same manner from 1,5-dichloroanthraquinone (commercial product).

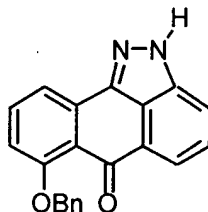
F. 5-Benzyloxyanthra[1,9cd]pyrazol-6(2H)-one

5



This compound may be made in the same manner from 1-nitro-4-benzyloxyanthraquinone. This starting material may be prepared as follows. Benzyl bromide is added to 1-nitro-4-hydroxyanthraquinone (Aldrich) and potassium carbonate in dimethylformamide, and the mixture is stirred for 16 hours. Water is added and the mixture is extracted with ethyl acetate (x2). The combined organic layer is washed sequentially with sodium bicarbonate solution, water, 1N hydrochloric acid, and brine, dried, and evaporated. The residue is chromatographed on silica gel to afford 1-nitro-4-benzyloxyanthraquinone.

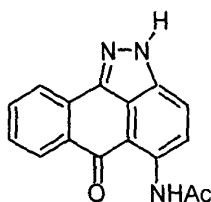
G. 7-Benzyloxyanthra[1,9cd]pyrazol-6(2H)-one



20

This compound may be made in the same manner from 1-nitro-5-benzyloxyanthraquinone, which starting material may prepared as disclosed in German Patent No. DE 2254199 to Reubke, Hohmann and Bien.

5 H. 5-(Acetylamino)anthra[1,9cd]pyrazol-6(2H)-one

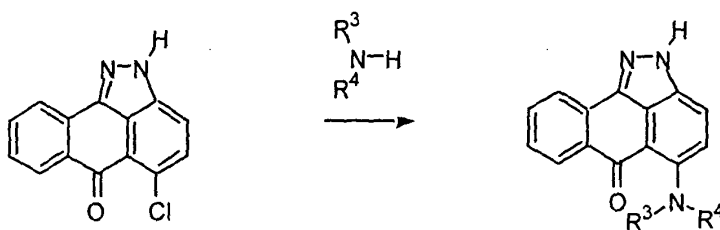


This compound may be made in the same manner from 4-acetylamino-1-chloroanthraquinone. This starting material may be prepared as follows. 4-Amino-1-chloroanthraquinone is taken in pyridine and treated with acetic anhydride. The mixture is stirred for 1 hour, and poured onto water. The solids are collected by filtration, washed with water, and dried in vacuo to give 4-acetylamino-1-chloroanthraquinone as a colorless solid.

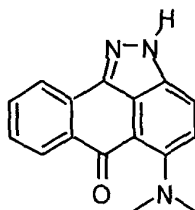
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EXAMPLE 2

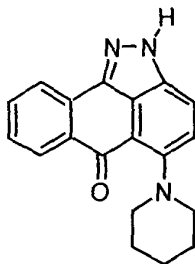
Synthesis of Representative Compounds



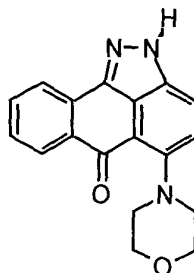
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A. 5-(Dimethylamino)anthra[1,9cd]pyrazol-6(2H)-one

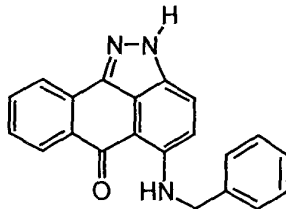
- 5 A mixture of 5-chloroanthra[1,9cd]pyrazol-6(2H)-one (Example 1-B) and dimethylamine in pyridine is heated at 100 °C for 16 hours. The mixture is cooled and evaporated. The residue is chromatographed on silica gel to give the desired compound as yellow solids.

10 B. 5-(1-Piperidiny)anthra[1,9cd]pyrazol-6(2H)-one

- 15 This compound may be made in the same manner using piperidine as the amine.

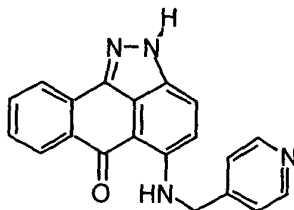
C. 5-(1-Morpholinyl)anthra[1,9cd]pyrazol-6(2H)-one

5 This compound may be made in the same manner using morpholine as the amine.

D. 5-(Benzylamino)anthra[1,9cd]pyrazol-6(2H)-one

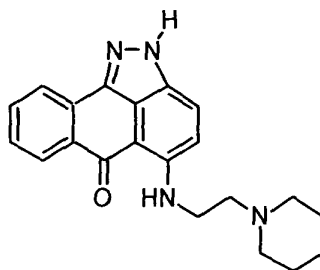
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This compound may be made in the same manner using benzylamine as the amine.

15 E. 5-[(4-Pyridylmethyl)amino]anthra[1,9cd]pyrazol-6(2H)-one

This compound may be made in the same manner using 4-pyridylmethylamine as the amine.

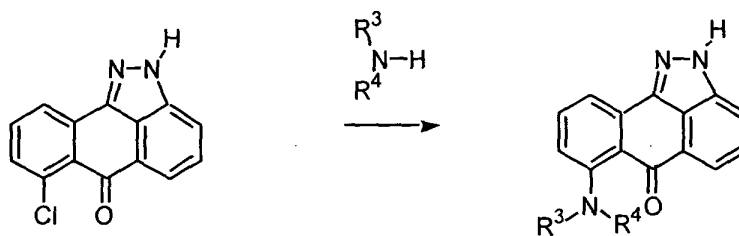
5 F. 5-{2-(1-Piperidiny)ethylamino}anthra[1,9cd]pyrazol-6(2H)-one

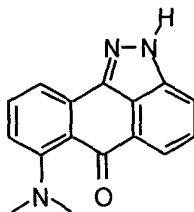


This compound may be made in the same manner using 2-(1-piperidyl)ethylamine as the amine.

EXAMPLE 3

Synthesis of Representative Compounds



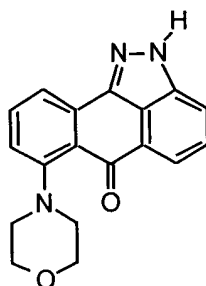
A. 7-(Dimethylamino)anthra[1,9cd]pyrazol-6(2H)-one

- 5 A mixture of 6-chloroanthra[1,9cd]pyrazol-6(2H)-one (Example 1-C) and dimethylamine in pyridine is heated at 100°C for 16 hours. The mixture is cooled and evaporated. The residue is chromatographed on silica gel to give the desired compound as yellow solids.

10 B. 5-(1-Piperidiny)anthra[1,9cd]pyrazol-6(2H)-one

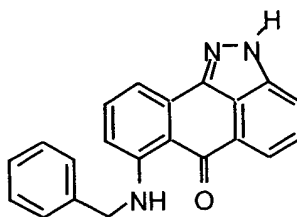
- 15 This compound may be made in the same manner using piperidine as the amine.

C. 5-(1-Morpholinyl)anthra[1,9cd]pyrazol-6(2H)-one



5 This compound may be made in the same manner using morpholine as the amine.

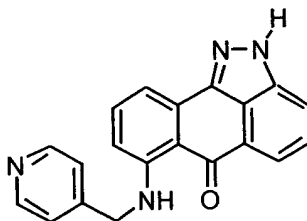
D. 5-(Benzylamino)anthra[1,9cd]pyrazol-6(2H)-one



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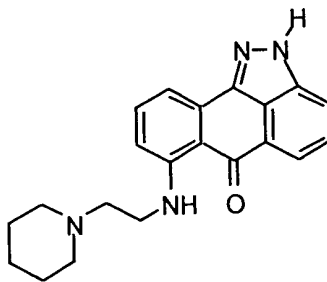
This compound may be made in the same manner using benzylamine as the amine.

15 E. 5-{{(4-Pyridyl)methyl}lamino}anthra[1,9cd]pyrazol-6(2H)-one



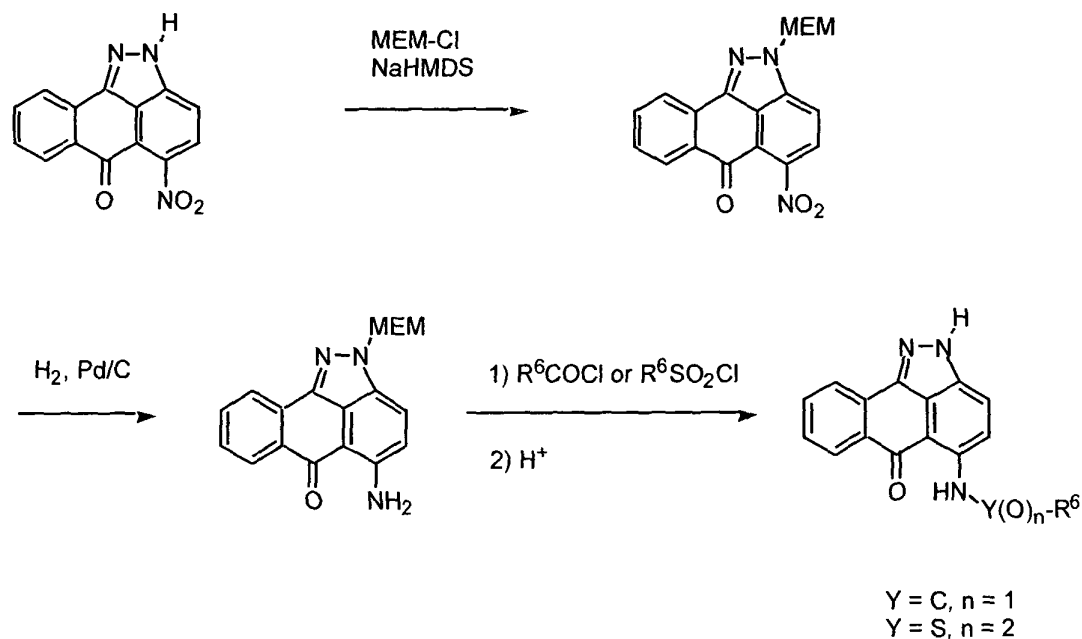
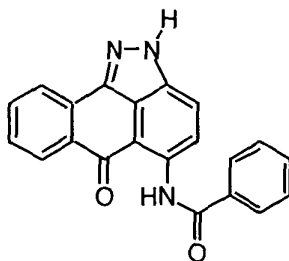
This compound may be made in the same manner using 4-pyridylmethylaniline as the amine.

5 F. 5-{2-(1-Piperidiny)ethylamino}anthra[1,9cd]pyrazol-6(2H)-one



This compound may be made in the same manner using 2-(1-piperidyl)ethylaniline as the amine.

EXAMPLE 4

Synthesis of Representative Compounds5 A. 5-(Benzoylamino)anthra[1,9cd]pyrazol-6(2H)-one

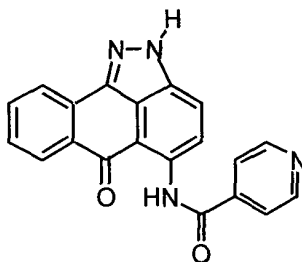
Benzoyl chloride is added to a solution of 2-(methoxyethoxymethyl)-5-
 10 aminoanthra[1,9cd]pyrazol-6-(2H)one and triethylamine in methylene chloride at 0°C. The mixture is stirred for 16 hours, quenched with water, and extracted with ethyl acetate (x2). The combined organic layer is washed with sodium bicarbonate solution, and brine, dried and evaporated. The crude reaction mixture is then taken in aqueous 6N hydrochloric acid, and heated at 80°C for 4 hours. After cooling, the mixture is extracted with ethyl acetate

(x2), washed with brine, dried, and evaporated. The residue is chromatographed on silica gel to furnish the desired amide as a yellow solid.

The starting material is prepared as follows. Sodium hexamethyldisilazide is added to a cooled (0°C) solution of 5-nitroanthra[1,9cd]pyrazol-6(2H)-one (Example 1-
 5 D) in tetrahydrofuran, and the mixture is stirred for 30 minutes at 0°C. MEM-chloride is added, and the mixture is stirred for 16 hours at room temperature. Water is added and the mixture is extracted with ethyl acetate (x2). The combined organic layer is washed with aqueous sodium bicarbonate solution, water, 1N hydrochloric acid, and brine, dried and evaporated. The residue is chromatographed on silica gel to give 2-MEM-5-
 10 nitroanthra[1,9cd]pyrazol-6(2H)-one as an oil.

Palladium(10%) on charcoal and 2-MEM-5-nitroanthra[1,9cd]pyrazol-6(2H)-one in ethanol is placed under 1-atm of hydrogen, and the mixture was stirred for 6 hours. The catalyst is filtered off over celite, and the filtrate is evaporated to dryness to give 2-(methoxyethoxymethyl)-5-aminoanthra[1,9cd]pyrazol-6-(2H)one, which is used
 15 without further purification.

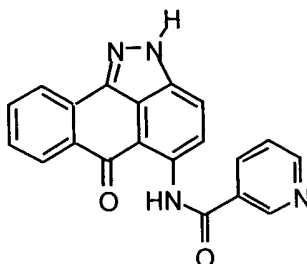
B. 5-(Isonicotinylamino)anthra[1,9cd]pyrazol-6(2H)-one



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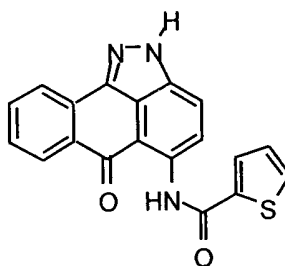
This compound may be made in the same manner using isonicotinoyl chloride as the acid chloride

C. 5-(Nicotinylamino)anthra[1,9cd]pyrazol-6(2H)-one



5 This compound may be made in the same manner using nicotinoyl chloride as the acid chloride.

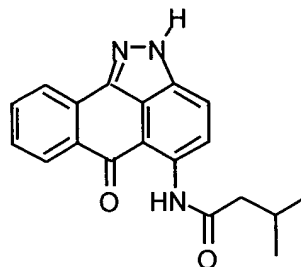
D. 5-(2-Thiophenecarbonylamino)anthra[1,9cd]pyrazol-6(2H)-one



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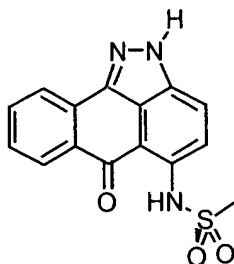
This compound may be made in the same manner using 2-thiophenecarboxylic acid as the acid chloride.

E. 5-(3-Methylbutyrylamino)anthra[1,9cd]pyrazol-6(2H)-one



5 This compound may be made in the same manner using isopentanoyl chloride as the acid chloride.

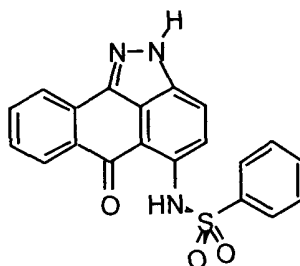
F. 5-(3-Methanesulfonylamino)anthra[1,9cd]pyrazol-6(2H)-one



10

This compound may be made in the same manner using methanesulfonyl chloride as the sulfonyl chloride.

G. 5-(3-Benzenesulfonylamino)anthra[1,9cd]pyrazol-6(2H)-one

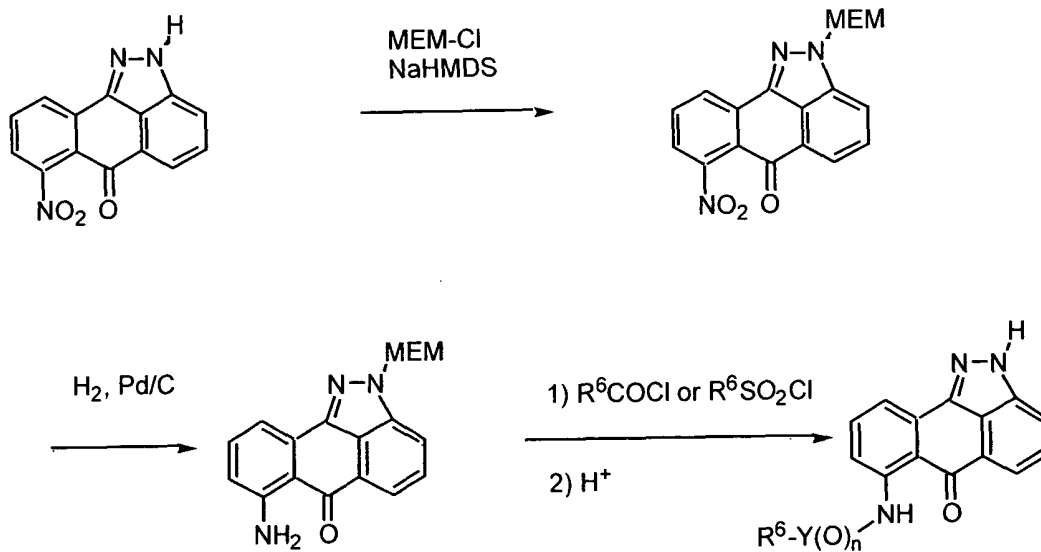


- 5 This compound may be made in the same manner using benzenesulfonyl chloride as the sulfonyl chloride.

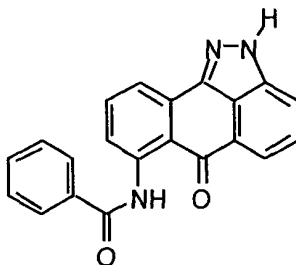
EXAMPLE 5

Synthesis of Representative Compounds

10



Y = C, n = 1
Y = S, n = 2

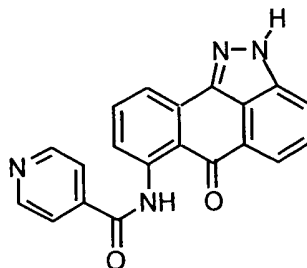
A. 7-(Benzoylamino)anthra[1,9cd]pyrazol-6(2H)-one

5 Benzoyl chloride is added to a solution of 2-(methoxyethoxymethyl)-7-aminoanthra[1,9cd]pyrazol-6-(2H)one and triethylamine in methylene chloride at 0°C. The mixture is stirred for 16 hours, quenched with water, and extracted with ethyl acetate (x2). The combined organic layer is washed with sodium bicarbonate solution, and brine, dried and evaporated. The crude reaction mixture is then taken in aqueous 6N hydrochloric acid, and heated at 80°C for 4 hours. After cooling, the mixture is extracted with ethyl acetate (x2), washed with brine, dried, and evaporated. The residue is chromatographed on silica gel to furnish the desired amide as a yellow solid.

The starting material is prepared as follows. Sodium hexamethyldisilazide is added to a cooled (0°C) solution of 7-nitroanthra[1,9cd]pyrazol-6(2H)-one (Example 1-
15 E) in tetrahydrofuran, and the mixture is stirred for 30 minutes at 0°C. MEM-chloride is added, and the mixture is stirred for 16 hours at room temperature. Water is added and the mixture is extracted with ethyl acetate (x2). The combined organic layer is washed with aqueous sodium bicarbonate solution, water, 1N hydrochloric acid, and brine, dried and evaporated. The residue is chromatographed on silica gel to give 2-MEM-7-nitroanthra[1,9cd]pyrazol-6(2H)-one as an oil.
20

Palladium(10%) on charcoal and 2-MEM-5-nitroanthra[1,9cd]pyrazol-6(2H)-one in ethanol is placed under 1-atm of hydrogen, and the mixture was stirred for 6 hours. The catalyst is filtered off over celite, and the filtrate is evaporated to dryness to give 2-(methoxyethoxymethyl)-7-aminoanthra[1,9cd]pyrazol-6-(2H)one, which is used
25 without further purification.

B. 7-(Isonicotinylamino)anthra[1,9cd]pyrazol-6(2H)-one

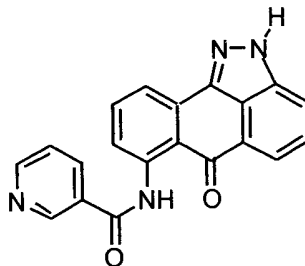


5

This compound may be made in the same manner using isonicotinoyl chloride as the acid chloride.

C. 7-(Nicotinylamino)anthra[1,9cd]pyrazol-6(2H)-one

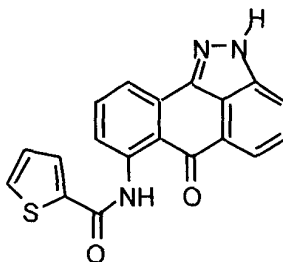
10



This compound may be made in the same manner using nicotinoyl chloride as the acid chloride.

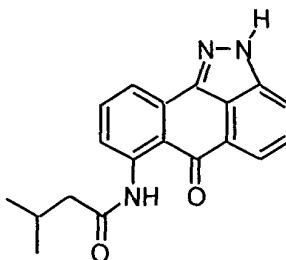
15

D. 5-(2-Thiophenecarbonylamino)anthra[1,9cd]pyrazol-6(2H)-one



5 This compound may be made in the same manner using 2-thiophenecarboxylic acid chloride as the acid chloride.

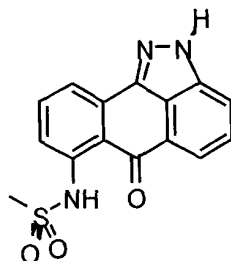
E. 7-(3-Methylbutyrylamino)anthra[1,9cd]pyrazol-6(2H)-one



10

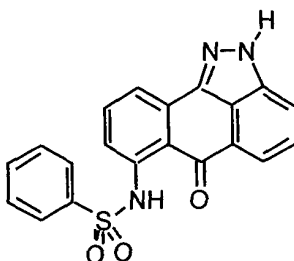
This compound may be made in the same manner using isopentanoyl chloride as the acid chloride.

F. 7-(3-Methanesulfonylamino)anthra[1,9cd]pyrazol-6(2H)-one



5 This compound may be made in the same manner using methanesulfonyl chloride as the sulfonyl chloride.

G. 7-(3-Benzenesulfonylamino)anthra[1,9cd]pyrazol-6(2H)-one

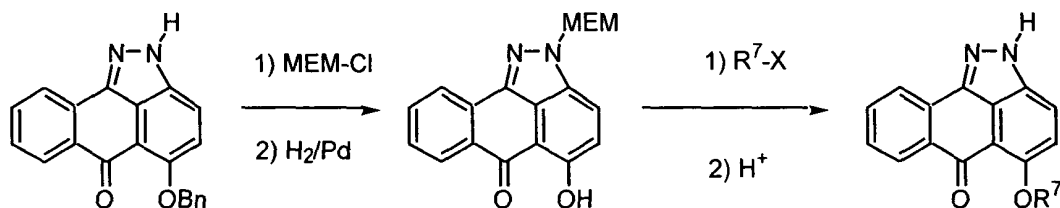


10

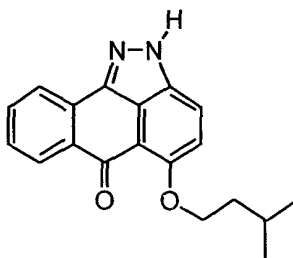
This compound may be made in the same manner using benzenesulfonyl chloride as the sulfonyl chloride.

15

EXAMPLE 6

Synthesis of Representative Compounds

5

A. 5-(3-Methylbutyloxy)anthra[1,9cd]pyrazol-6(2H)-one

- 10 Isopentyl bromide is added to a mixture of 3-(2-methoxyethoxymethyl)5-hydroxyanthra[1,9cd]pyrazol-6(2H)-one and potassium carbonate in dimethylformamide at room temperature. After stirring the mixture for sixteen hours, water is added, and the mixture was extracted with ethyl acetate (x2). The combined organic layer is washed with aqueous sodium bicarbonate, water, 1N hydrochloric acid, and brine, dried and evaporated.
- 15 The residue is taken in 6N hydrochloric acid and heated at 80°C for 4 hours. After cooling, the mixture is extracted with ethyl acetate (x2), and the combined organic layer is washed with brine, dried, and evaporated. The residue is purified by column chromatography to afford the title compound as yellow solid.

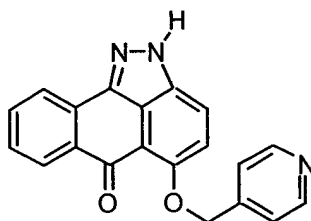
20 The starting material is prepared as follows. Sodium hexamethyldisilazide is added to a cooled (0°C) solution of 5-benzyloxanthra[1,9cd]pyrazol-6(2H)-one (Example 1-F) in tetrahydrofuran, and the mixture is stirred for 30 minutes at 0°C. MEM-chloride is added, and the mixture is stirred for 16 hours at room temperature. Water is

added and the mixture is extracted with ethyl acetate (x2). The combined organic layer is washed with aqueous sodium bicarbonate solution, water, 1N hydrochloric acid, and brine, dried and evaporated. The residue is chromatographed on silica gel to give 2-MEM-5-benzyloxyanthra[1,9cd]pyrazol-6(2H)-one as an oil.

- 5 Palladium(10%) on charcoal and 2-MEM-5-benzyloxyanthra[1,9cd]pyrazol-6(2H)-one in ethanol is placed under 1-atm of hydrogen, and the mixture stirred for 6 hours. The catalyst is filtered off over celite, and the filtrate is evaporated to dryness to give 2-(2-methoxyethoxymethyl)-5-hydroxyanthra[1,9cd]pyrazol-6-(2H)one, which is used without further purification.

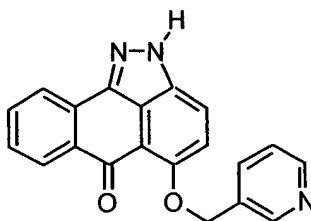
10

- B. 5-(4-Pyridylmethoxy)anthra[1,9cd]pyrazol-6(2H)-one



- 15 This compound may be made in the same manner using chloromethyl-4-pyridine as the alkyl halide.

- C. 5-(3-Pyridylmethoxy)anthra[1,9cd]pyrazol-6(2H)-one

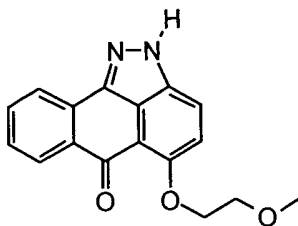


20

This compound may be made in the same manner using chloromethyl-3-pyridine as the alkyl halide.

D. 5-(2-Methoxyethoxy)anthra[1,9cd]pyrazol-6(2H)-one

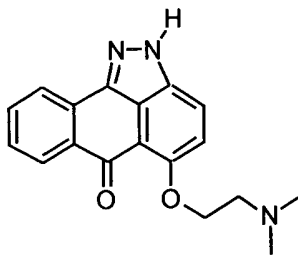
5



This compound may be made in the same manner using 2-methoxyethyl bromide as the alkyl halide.

10

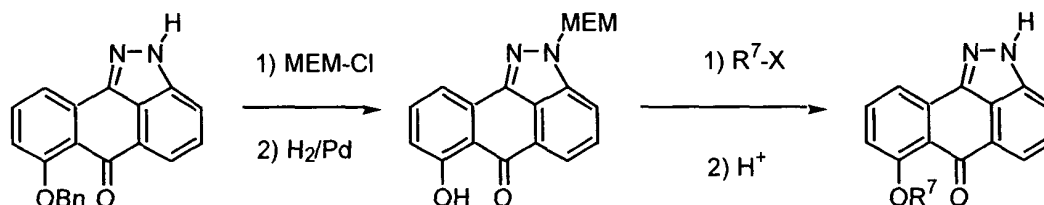
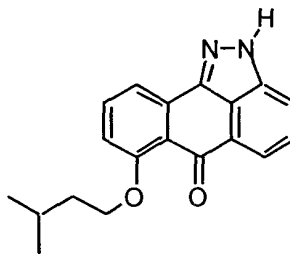
E. 5-(2-Dimethylaminoethoxy)anthra[1,9cd]pyrazol-6(2H)-one



15

This compound may be made in the same manner using 2-dimethylaminoethyl chloride as the alkyl halide.

EXAMPLE 7

Synthesis of Representative CompoundsA. 7-(3-Methylbutyloxy)anthra[1,9cd]pyrazol-6(2H)-one

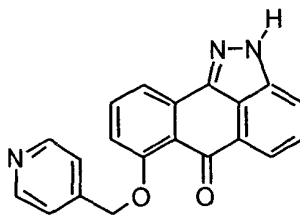
Isopentyl bromide is added to a mixture of 3-(2-methoxyethoxymethyl)-7-hydroxyanthra[1,9cd]pyrazol-6(2H)-one and potassium carbonate in dimethylformamide at room temperature. After stirring the mixture for sixteen hours, water is added, and the mixture was extracted with ethyl acetate (x2). The combined organic layer is washed with aqueous sodium bicarbonate, water, 1N hydrochloric acid, and brine, dried and evaporated. The residue is taken in 6N hydrochloric acid and heated at 80°C for 4 hours. After cooling, the mixture is extracted with ethyl acetate (x2), and the combined organic layer is washed with brine, dried, and evaporated. The residue is purified by column chromatography to afford the title compound as yellow solid.

The starting material is prepared as follows. Sodium hexamethyldisilazide is added to a cooled (0°C) solution of 7-benzyloxanthra[1,9cd]pyrazol-6(2H)-one (Example 1-F) in tetrahydrofuran, and the mixture is stirred for 30 minutes at 0°C. MEM-

chloride is added, and the mixture is stirred for 16 hours at room temperature. Water is added and the mixture is extracted with ethyl acetate (x2). The combined organic layer is washed with aqueous sodium bicarbonate solution, water, 1N hydrochloric acid, and brine, dried and evaporated. The residue is chromatographed on silica gel to give 2-MEM-7-benzyloxyanthra[1,9cd]pyrazol-6(2H)-one as an oil.

Palladium(10%) on charcoal and 2-MEM-7-benzyloxyanthra[1,9cd]pyrazol-6(2H)-one in ethanol is placed under 1-atm of hydrogen, and the mixture was stirred for 6 h. The catalyst is filtered off over celite, and the filtrate is evaporated to dryness to give 2-(2-methoxyethoxymethyl)-7-hydroxyanthra[1,9cd]pyrazol-6-(2H)one, which is used without further purification.

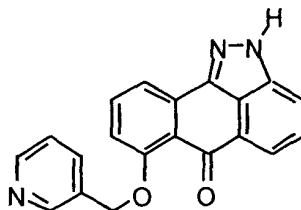
B. 7-(4-Pyridylmethoxy)anthra[1,9cd]pyrazol-6(2H)-one



15

This compound may be made in the same manner using chloromethyl-4-pyridine as the alkyl halide.

C. 7-(3-Pyridylmethoxy)anthra[1,9cd]pyrazol-6(2H)-one

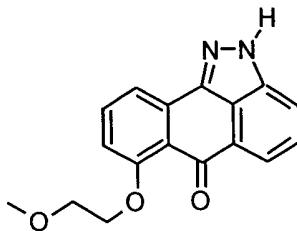


20

This compound may be made in the same manner using chloromethyl-3-pyridine as the alkyl halide.

D. 7-(2-Methoxyethoxy)anthra[1,9cd]pyrazol-6(2H)-one

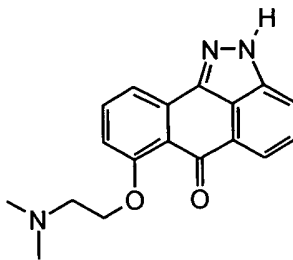
5



This compound may be made in the same manner using 2-methoxyethyl bromide as the alkyl halide.

10

E. 7-(2-Dimethylaminoethoxy)anthra[1,9cd]pyrazol-6(2H)-one



15

This compound may be made in the same manner using 2-dimethylaminoethyl chloride as the alkyl halide.

EXAMPLE 8

Activity of Representative Compound

The compounds of this invention may be assayed for their activity
5 accordingly to the following procedures.

JNK Assay

To 10 μ L of the test compound in 20% DMSO/80% dilution buffer consisting of 20 mM HEPES (pH 7.6), 0.1 mM EDTA, 2.5 mM magnesium chloride,
10 0.004% Triton x100, 2 μ g/mL leupeptin, 20 mM β -glycerolphosphate, 0.1 mM sodium vanadate, and 2 mM DTT in water is added 30 μ L of 50-200 ng His6-JNK1, JNK2 or JNK3 in the same dilution buffer. The mixture is preincubated for 30 minutes at room temperature. Sixty microliter of 10 μ g GST-c-Jun(1-79) in assay buffer consisting of 20 mM HEPES (pH 7.6), 50 mM sodium chloride, 0.1 mM EDTA, 24 mM magnesium
15 chloride, 1 mM DTT, 25 mM PNPP, 0.05% Triton x100, 11 μ M ATP, and 0.5 μ Ci γ -32P ATP in water is added and the reaction is allowed to proceed for 1 hour at room temperature. The c-Jun phosphorylation is terminated by addition of 150 μ L of 12.5% trichloroacetic acid. After 30 minutes, the precipitate is harvested onto a filter plate, diluted with 50 μ L of the scintillation fluid and quantified by a counter. The IC₅₀ values
20 are calculated as the concentration of the test compound at which the c-Jun phosphorylation is reduced to 50% of the control value. Preferred compounds of the present invention have an IC₅₀ value ranging 0.01 - 10 μ M in this assay. To this end, a preferred compound of this invention is Compound 1, which has an IC₅₀ according to this assay of 0.11 μ M for JNK1 and JNK2, and 0.15 μ M for JNK3.

25

Selectivity For JNK

Compound 1 was also assayed for its inhibitory activity against the following protein kinases by techniques known to those skilled in this field (*see, e.g., Protein Phosphorylation*, Sefton & Hunter, Eds., Academic Press, pp. 97-367, 1998):

<u>Enzyme</u>	<u>IC₅₀</u>
p38-2	>30,000 nM
ERK1	>30,000 nM
MEKK1	>30,000 nM
IKK1	>30,000 nM
IKK2	>30,000 nM
PKA	>30,000 nM
PKC	>10,000 nM
EGF-TK	>10,000 nM

Jurkat T-cell Il-2 Production Assay

- Jurkat T cells (clone E6-1) are purchased from the American Tissue Culture
- 5 Collection and maintained in growth media consisting of RPMI 1640 medium containing 2 mM L-glutamine (Mediatech), with 10% fetal bovine serum (Hyclone) and penicillin/streptomycin. All cells are cultured at 37°C in 95% air and 5% CO₂. Cells are plated at a density of 0.2 x 10⁶ cells per well in 200 µL of media. Compound stock (20 mM) is diluted in growth media and added to each well as a 10x concentrated solution in a
- 10 volume of 25 µL, mixed, and allowed to pre-incubate with cells for 30 minutes. The compound vehicle (dimethylsulfoxide) is maintained at a final concentration of 0.5% in all samples. After 30 minutes the cells are activated with PMA (phorbol myristate acetate; final concentration 50 ng/mL) and PHA (phytohemagglutinin; final concentration 2 µg/mL). PMA and PHA are added as a 10x concentrated solution made up in growth
- 15 media and added in a volume of 25 µL per well. Cell plates are cultured for 10 hours. Cells are pelleted by centrifugation and the media removed and stored at -20 °C. Media aliquots are analyzed by sandwich ELISA for the presence of IL-2 as per the manufacturers instructions (Endogen). The IC₅₀ values are calculated as the concentration of the test compound at which the Il-2 production was reduced to 50% of the control value. Preferred

compounds of the present invention have an IC_{50} value ranging 0.1 - 30 μM in this assay. Figure 1 presents the dose dependent inhibition of IL-2 in Jarkat T-Cells by Compound 1 according to this procedure, with a resulting IC_{50} of 5 μM

5 Mouse *in vivo* LPS-Induced TNF- α Production Assay

Non-fasted mice are acclimatized for at least 7 days. Groups of 4 to 6 female BALB/c or CD-1 mice (8-10 weeks of age from Charles River laboratories) are pretreated with test compound, either by intravenous injection or by oral gavage 15 – 180 minutes prior to the injection of 0.5 mg/kg Bacto LPS from *E. coli* 055:B5 (Difco Labs).

10 Ninety minutes after LPS challenge, a terminal bleed is performed via abdominal vena cava and blood is allowed to clot at room temperature for 30 minutes in Microtainer serum separator tubes. After separation by centrifugation, the serum is stored frozen at $-80^{\circ}C$. ELIZA is performed on thawed, diluted samples (1:10 to 1:20) using a Mouse TNF-alpha kit (Biosource International). The ED_{50} values are calculated as the dose of the test

15 compound at which the TNF- α production is reduced to 50% of the control value. Preferred compounds of the present invention have an ED_{50} value ranging 1 - 30 mg/kg in this assay. Figure 2 illustrates the results of this experiment utilizing Compound 1 administered by intravenous injection (I.V.) at 15 and 30 mg/kg, as well as by per os (P.O.) at 7.5, 15 and 30 mg/kg. Vehicle alone (PEG-400, propylene glycol, cremophor EL, and

20 ethanol in normal saline, "PPCES") and dexamethasone-21 acetate ("DEX") (1 mg/kg P.O.) were run as controls ($n = 6$, $* = p 0.01$). Compound 1 was administered 15 minutes pre-LPS challenge, and bleed occurred 90 minutes post LPS.

Inhibition of Leukocyte Recruitment in Rat Inflamed Lung

25 Aerosol administration of ovalbumin in Brown Norway Rats previously sensitized by injection of ovalbumin (OA) results in an allergic airway inflammation marked by the generation of an eosinophil- and T-lymphocyte-rich leukocytic infiltration in the lungs (*see* Richards et al., *Am. J. Physiol*, 271:2 Pt 1, L267-76, 1996). Compound 1 was administered by subcutaneous injection at a dose of 30 mg/kg, b.i.d. for 3 days prior to

ovalbumin challenge by aerosol. Cells counts were obtained from samples of broncho-alveolar lavage, the results of which are illustrated in Figure 3 (V = PPCES vehicle).

Rat *In Vivo* Adjuvant Arthritis

5 Male Lewis rats were immunized with complete Freund's adjuvant on day 0 to induce an aggressive arthritis characterized by joint destruction and paw swelling. Compound 1 was administered subcutaneously once daily from day 8 to day 20. Paw swelling was determined by water displacement plethysmometry (see Figure 4A; * = $p < 0.01$). Radiographs were obtained of the right hind paw to assess bone changes using a
10 semi-quantitative scoring system: demineralization (0-2+), calcaneal erosion (0-1+), and heterotopic bone formation (0-1+), with a maximum possible score = 6 (see Figure 4B). Activation of AP-1 (see Figure 4C) was determined by DNA binding activity in an electrophoretic mobility shift assay (EMSA) (Ausubel et al., *Short Protocols in Molecular Biology*, Second Edition, John Wiley & Sons Publisher, New York, 1992). Matrix
15 metalloproteinase-13 expression (see Figure 4D) was measured by northern blot analysis of MMP-13 mRNA (Ausubel et al., *supra*) (see also Winter et al., *Arthritis and Rheumatism* 9(3):394-404, 1966; Weichman et al., *Pharmacological Methods in the Control of Inflammation*, Chang and Lewis Eds., Alan R. Liss, Inc., Publ., New York, 1989).

20 Kainic Acid-Induced Seizure Response

Compound 1 was administered to male CD rats at 10 mg/kg intravenously through a tail vein catheter. This was followed immediately by a 30 mg/kg subcutaneous injection. Vehicle controls received the same injection volumes of the PPCES vehicle alone. Thirty minutes later, animals were given a 1- mg/kg i.p. injection of kainic acid in
25 normal saline solution. This dose of kainic acid has been previously reported to induce a seizure syndrome in rats (Maj et al., *Eur. J. Pharm.* 359:27-32, 1992). Seizure behavior was monitored for 4 hours following kainic acid injection. As presented in Figure 5, behaviors were assessed based on the following cumulative scoring system: 1 pt. = arrest of motion; 2 pts. = myoclonic jerks of the head and neck (moderate); 3 pts. = unilateral or

5 It will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without departing from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.